Influence of experimental venue on phenotype: multiple traits reveal multiple answers

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Abstract: 1. Experiments in ecology occur in the laboratory, mesocosm, or field. The choice of venue can influence the outcome and may be associated with trade-offs involving realism and precision. 2. We evaluated these trade-offs in an experiment measuring effects of venue on larval traits of Rana temporaria tadpoles. The design included lab, mesocosm, and field venues, crossed with two treatments (presence and absence of caged Anax imperator dragonfly larvae). Realism of venues was evaluated by comparing experimental with wild tadpoles. 3. Venue influenced nearly every trait we measured, but some were more sensitive to venue than others. Larval and metamorphic performance, external morphology, and predator-induced plasticity in many traits varied among venues, while behavior was less dependent on venue. Tadpoles in mesocosms were most similar to those in field enclosures and the wild, although the phenotypic response to predation risk was greatest in the mesocosm venue. The laboratory environment triggered highly distinctive morphology. Precision was not higher in the laboratory than in other venues. 4. This study suggests that both constraints and research questions must be considered when choosing an appropriate experimental venue.

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Influence of experimental venue on phenotype: multiple traits reveal multiple answers

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Summary

1. Experiments in ecology occur in the laboratory, mesocosm, or field. The choice of venue can influence the outcome and may be associated with trade-offs involving realism and precision.

2. We evaluated these trade-offs in an experiment measuring effects of venue on larval traits of *Rana temporaria* tadpoles. The design included lab, mesocosm, and field venues, crossed with two treatments (presence and absence of caged *Anax imperator* dragonfly larvae). Realism of venues was evaluated by comparing experimental with wild tadpoles.

3. Venue influenced nearly every trait we measured, but some were more sensitive to venue than others. Larval and metamorphic performance, external morphology, and predator-induced plasticity in many traits varied among venues, while behavior was less dependent on venue. Tadpoles in mesocosms were most similar to those in field enclosures and the wild, although the phenotypic response to predation risk was greatest in the mesocosm venue. The laboratory environment triggered highly distinctive morphology. Precision was not higher in the laboratory than in other venues.

4. This study suggests that both constraints and research questions must be considered when choosing an appropriate experimental venue.

Key-words: behavior, development, field experiment, laboratory experiment, mesocosm experiment, morphology, tadpole, *Rana*

Introduction

Discussions about experimental venue have been a part of ecology for decades (Diamond 1986; Hairston 1989). The main focus has been a series of well-accepted trade-offs associated with field and laboratory venues, involving realism, control, precision, and replication. However, as the topic of venue itself has become an object of experimental study, some of the old paradigms have been overturned and new insights into the relevant trade-offs have emerged. For example, controlled experiments in laboratory containers or mesocosms do not in general yield larger effect sizes than field studies, and lab experiments do not necessarily have greater precision (Weigensberg & Roff 1996; Skelly & Kiesecker 2001; Bancroft, Baker & Blaustein 2007). On the other hand, it does appear that experimental designs implemented under more controlled mesocosm or lab conditions are more complex and better replicated, and effect sizes can be larger in the laboratory for certain types of manipulations (Skelly & Kiesecker 2001; Bell, Neill & Schluter 2003).

Some of these insights emerge from reviews and meta-analyses of experiments employing different kinds of venues (Petersen, Cornwell & Kemp 1999; Skelly & Kiesecker 2001; Bell *et al.* 2003; Bancroft *et al.* 2007). One problem with this approach is that differences among venues in the original experiments are confounded with other features that vary among studies, including organisms, geographical localities, investigators, and numerous methodological details (Chalcraft, Binckley & Resetarits 2005). What is needed are explicit manipulations of venue that hold all else constant as far as possible (Skelly 2002). Our goal here was to gauge the effects of three types of venue on larval phenotypes of *Rana temporaria*, a European amphibian that is a frequent research subject in experimental ecology. We observed behavior, external morphology, larval and metamorphic performance, and plasticity in these traits between two experimental treatments (presence and absence of predation risk), carried out in three different experimental venues. Our results are important for interpreting the ecological literature, for choosing appropriate venues in future studies, and for weighing trade-offs among realism, control, precision, and replication.

Materials and methods

The experiment had a complete factorial design consisting of three venues crossed with two treatments. The treatments were presence and absence of non-lethal dragonfly larvae, and the three venues were laboratory, outdoor mesocosm, and field enclosure. Each of the three venue types was represented by two independent realizations of the venue, here called "settings" and treated as an additional factor nested within venue (Table 1). The two laboratory settings were small plastic bins (volume 1.1 L, containing 1 tadpole) and large plastic bins (5.2 L, 5 tadpoles). In the mesocosm venue, the two settings were small plastic tubs (80 L, 12 tadpoles) and large fiberglass stock tanks (675 L, 60 tadpoles). The two field settings consisted of mesh enclosures (200 L, 35 tadpoles) placed in two different natural ponds. We also included a non-experimental sample used to evaluate the realism of the experimental venues: wild tadpoles collected in the source pond from which the experimental tadpoles originated and in the two ponds used in the field enclosure venue.

The impact of setting may vary among venues. In laboratory and mesocosm venues, setting corresponds to differences in volume and surface area, both of which are known to influence the performance of aquatic organisms (Pearman 1993; Petersen *et al.* 1999; Spivak, Vanni & Mette 2011). In the field enclosure venue, setting reflects a difference between ponds that are shaded to different degrees, and this in turn can affect numerous traits of amphibian larvae (Skelly, Freidenburg & Kiesecker 2002; Schiesari 2006; Van Buskirk 2011). Thus, settings are not directly comparable across venues, and should be interpreted as representing different venue types that often occur in ecological experimentation. This can be viewed as a strength of the design, because repeating the experiment in multiple settings improves the generality of our conclusions.

We designed protocols to reflect methods currently used by experimentalists studying amphibians in laboratory, mesocosm, and field experiments (e.g., Skelly 2002; Fraker *et al.* 2009). Laboratory bins contained aged tap water in a room maintained at 20 °C on a 12:12 light:dark cycle. Three times per week we siphoned out half the water and replaced it; on two occasions we renewed all the water in the bins. Two types of water were used to replenish the bins, and this allowed us to manipulate apparent predation risk. Half the bins received water from an 80 L tub in which two *Anax imperator* dragonfly larvae had each been fed 300 mg of *Rana temporaria* tadpoles, three times per week. These bins therefore contained waterborne dragonfly kairomones and tadpole alarm chemicals, which are frequently used to simulate predation risk in laboratory experiments (Kraft, Franklin & Blows 2006; Urban 2008; Fraker *et al.* 2009). Bins in the no-predator treatment received aged tap water. Tadpoles were fed three times per week with a 4:1 ratio of finely-ground rabbit food and Tetramin fish flakes. The quantity of food was adjusted continuously so that tadpoles received approximately 20% of their mass per day.

The mesocosms were arranged in a field at the University of Zurich, Switzerland, filled with tap water 27 days before the experiment began, and kept covered with lids constructed of 43% shade cloth. We stocked each mesocosm with dried leaf litter (40 g in the 80 L setting and 400 g in the 675 L setting) and rabbit chow (2 g in 80 L tubs and 10 g in 675 L tanks), and made several additions of water and zooplankton collected from a nearby pond. No supplemental food was added during the experiment. Non-lethal predation risk was manipulated by placing floating cages containing an *A. imperator* larva within half the mesocosms (1 cage in the 80 L setting, 2 cages in the 675 L setting). The cages were about 1 L in volume, constructed of an 11 cm length of plastic tubing with window screen on the ends. We fed each predator 300 mg of tadpoles three times per week; this produced a concentration of waterborne kairomones in the 80 L mesocosms identical to that in the laboratory venue (Table 1). Predator-free mesocosms contained empty cages.

The field enclosure venue consisted of two sets of six enclosures in each of two different ponds, chosen to represent typical conditions under which field experiments on larval amphibians are conducted. One pond, here called the “sunny” pond, has a muddy substrate with 87% coverage by submerged aquatic vegetation and a canopy cover of 9.5%, measured as the obstructed sun arc between 10:15 and 17:00 local time in late April. The sunny pond is 13.7 km N of Zurich (47.49086 N, 8.53638 E). The second pond, called the “shady” pond, is 3 km S of the sunny pond (47.46484 N, 8.53542 E) and has a substrate of decomposing leaf litter, 15% coverage by emergent aquatic vegetation, and 60% canopy cover. Both ponds contained numerous predators, including adult *Notonecta glauca* backswimmers, larval *Aeshna cyanea* and *A. imperator* dragonflies, and larval *Dytiscus marginalis* beetles. The enclosures had a surface area of 1 m^2 and were constructed of fiberglass window screen (0.5 mm mesh) covering a wooden frame, reinforced with hardware cloth on

the bottom and covered with a lid of 43% shade cloth. The substrate was a mixture of leaf litter, vegetation, and mud that we scooped from 1 m^2 of the pond immediately adjacent to the enclosure and searched to remove any potential predators. We set the enclosures initially at a depth of about 30 cm, but the water level declined during the experiment so that the average volume was 200 L. The caged-predator treatment contained a single caged *A. imperator* larva, which was fed 300 mg *R. temporaria* tadpoles three times per week. The no-predator enclosures contained an empty cage. All cages were rotated among pools or enclosures within treatments on each feeding event to even out potential differences among individual dragonflies.

Experimental units were arranged in spatial blocks in all six settings, and treatments were assigned at random within blocks. Replication and other experimental details are summarized in Table 1.

The experimental animals came from seven clutches of *R. temporaria* eggs collected from a pond 1.3 km SSE of the sunny pond (47.48103 N, 8.54500 E). We initiated the experiment on 6 April 2009, when the tadpoles were two days old (stage 23; Gosner 1960). Each clutch contributed an equal number of individuals to every experimental unit as far as possible. For example, each of the field enclosures received 5 tadpoles from every clutch, and the 675 L mesocosms received 8 tadpoles from each of three clutches and 9 from the remaining four clutches. In the 1 L laboratory setting, four clutches contributed one replicate each and three clutches contributed two replicates. Tadpoles remained in their assigned bins, tubs, or tanks until they reached stage 42 (forelimb emergence), with the exception of those in field enclosures. In that venue, we transferred all tadpoles to 80 L mesocosms on campus just after the first individuals reached metamorphosis, because we were unable to reliably collect metamorphs in the complex substrate of the enclosures. The impact on the results of bringing tadpoles to campus was probably minor, because the average individual spent only 4.3 d in the mesocosms before metamorphosing (6% of the larval period). The experiment continued, and metamorphs were collected daily, until all individuals reached stage 45.

MEASURING TRAITS

Life history traits. – We recorded body mass and developmental stage on 28 April, when tadpoles were 24 days old. This included all animals in the laboratory experiment, a subsample of those in the mesocosms (6 per 80 L tub, 8 per 675 L tank) and field venue (10 per enclosure), and samples of wild tadpoles taken from the experimental ponds (10 tadpoles per pond) and the pond where egg clutches were originally collected (9 tadpoles). Wild tadpoles were collected by dip-netting for a few minutes in each of several parts of the pond, and immediately returning individuals with no visible tail damage to the lab for measurement. We weighed all tadpoles and determined developmental stages from photographs of each individual. Metamorphic performance was represented by survival, mass, and age at stage 45.

Behavior. – We observed behavior in the laboratory and mesocosm venues on 22 April, when tadpoles were 18 days old. Each experimental unit was visited repeatedly, and the number of visible animals that were active (swimming or feeding) and inactive (resting) was counted. The 675 L tanks were visited 6 times during the day, the 80 L tubs 11 times, and both 1 L and 5 L laboratory bins 19 times. Tadpoles not visible to the investigator in the mesocosms were recorded as hiding in the leaf litter. We estimated the number alive in each mesocosm on 22 April assuming constant per capita daily mortality. There were two alternative measures of activity in mesocosms. If we assumed that hiding animals were inactive, activity was the number active divided by the estimated number alive, which probably underestimates true activity. The other measure of activity, the number active divided by the number observed, effectively assumed that hiding and visible tadpoles were equally active, which overestimates activity. Behavioral data were not collected in the field enclosures because tadpoles could not be seen.

Morphology. – We used the photographs from 28 April to measure morphological shape of all tadpoles in the laboratory experiment, 10 tadpoles per enclosure in the field venue, 6 tadpoles per 80 L mesocosm, 8 tadpoles per 675 L mesocosm, and a total of 29 wild tadpoles. Each image had lateral and ventral views of the tadpole in a water-filled Plexiglas chamber. Animals were returned to their experimental unit or pond after photography.

We used geometric morphometric analyses to describe variation in tadpole shape. Geometric methods correct for differences in size, location, and orientation between specimens, and use the

relative positions of landmarks to quantify shape (Zelditch *et al.* 2004). We used the image analysis program ImageJ to place 22 side-view landmarks and 13 bottom-view landmarks on each photograph (defined in Van Buskirk 2011). Specimens were scaled to unit size and rotated to a common orientation using Procrustes superimposition. We then projected the landmarks back into Euclidean space and subjected them to Principal Components Analysis, and retained the most important components (termed relative warps, RWs) to describe variation in shape. The first four RWs were included for the lateral view, comprising 83.4% of all shape variation; three RWs were included for the ventral view, comprising 85.5% of variation. Illustrations of the RWs generated by a thin plate spline algorithm are in Supporting Information A. Lateral and ventral RWs were in some cases correlated with each other. For example, lateral RW1 was positively correlated with ventral RW1 (r = 0.80, N = 350 tadpoles), because both RWs represent a short tail and large head/body. Ventral RW1 was also correlated with lateral RW2 (r = -0.49), reflecting an association between a wide and deep head/body, especially in the gut region, and a deep anterior part of the tail.

ANALYSES

Analyses of variance evaluated the influence of venue, setting nested within venue, predator treatment, and their interactions on tadpole phenotypes. We began with multivariate analyses for performance traits (body mass and developmental stage at 24 days, survival to metamorphosis, mass and age at metamorphosis) and morphological shape (the 7 RWs). Masses were log transformed before analysis, and survival and behavioral traits were arcsine square root transformed. Supporting Information D reports results of a parallel set of analyses on a set of size-corrected length measures, included for comparison with earlier studies of amphibian morphology.

To evaluate the precision of estimated trait values in each of the settings, we calculated average coefficients of variation (CV) among replicates for life history and behavior, and variance among replicates was measured for shape components. The CV is undefined for relative warps, which have an average value of zero.

Results

Life history. – Venue and setting strongly influenced the five life history traits in a multivariate analysis of variance (venue: Wilks' $F_{10,44} = 70.9$, $P < 0.0001$; setting nested within venue: Wilks' $F_{15,61} = 3.48$, $P = 0.0003$). Univariate tests revealed that venue had significant effects on all traits, whereas setting was important only for age and size at metamorphosis (Table 2). Tadpoles raised under laboratory conditions developed fastest and were frequently the heaviest (Fig. 1). The field venue led to decreases in all fitness related traits: tadpoles developed slowly and metamorphosed at small sizes in the enclosures. Survival was lowest in the field enclosures, for unknown reasons (shady pond: 0.650; sunny pond: 0.837), and approximately equal in the laboratory and mesocosm venues (ranging from 0.90 to 0.97). Wild tadpoles from the sunny and shady ponds were similar in size and developmental stage to experimental tadpoles raised in the same pond (Fig. 1A, B). Tadpoles from the source pond were larger and more developmentally advanced than those in the other two ponds.

There was no effect of the caged-dragonfly treatment on life history (MANOVA; Wilks' $F_{5,22} = 1.76$, $P = 0.1631$), but Fig. 1 and the univariate analyses in Table 2 suggest that predators caused somewhat reduced early growth and delayed development at both tadpole and metamorph stages. More important was the highly significant treatment-by-venue interaction (Wilks' $F_{10,44} = 4.82$, $P = 0.0001$). Predation risk induced larger body size at metamorphosis in the laboratory, but slightly smaller size in the field (Fig. 1C). Metamorphosis was delayed by at least a week in mesocosms when predators were present, but was if anything accelerated by predation risk in the laboratory (Fig. 1D). Interactions between treatment and setting (nested within venue) for body size arose because tadpoles in the 1L lab bins grew especially large under predation risk, and tadpoles in the 80L mesocosms were especially small with predators (Fig. 1A, C).

Behavior. – Activity was mostly influenced by treatment and to a lesser extent by venue (Fig. 2, Table 2). The comparison between mesocosm and laboratory venues depended on assumptions about the behavior of invisible animals in mesocosms. If hiding animals were ignored, effectively assuming that they behave the same as those that were visible, then activity was greatly reduced in the lab in the absence of predators (Fig. 2A). On the other hand, if we assume that hiding animals were not moving, then activity was higher in the lab under predation risk (Fig. 2B). The dragonfly treatment caused

somewhat lower activity in both venues, but the significant treatment-by-venue interaction indicated that the response in the lab was comparatively weak. In mesocosms, tadpoles exposed to predators reduced their activity by at least 70%, and in the 80 L setting fully 100% of individuals were either resting or hiding. Supporting Information B shows results for the proportion of individuals feeding, swimming, and hiding in the litter.

Morphology. – Morphological shape was highly sensitive to venue and setting (MANOVA on seven relative warps; venue: Wilks' $F_{14,40} = 51.5$, $P < 0.0001$; setting: Wilks' $F_{21,58} = 3.97$, $P = 0.0001$). Laboratory tadpoles had a relatively long and shallow tail with a short and narrow head/body and reduced gut mass (lateral RW1 and ventral RW1; Fig. 3, Table 2). On most measures, animals in mesocosms were similar to those in field enclosures and the wild, although they were intermediate on lateral RW1 (Supporting Information C).

The caged-predator treatment induced numerous changes in shape (Wilks' $F_{7,20} = 20.5$, $P < 0.0001$), especially in the depth of the tail, attachment of the dorsal fin, relative gut mass, and orientation of the mouth and eyes (lateral RW3 and RW4; Fig. 3 and Supporting Information C). These responses were highly variable among venues (treatment-by-venue interaction: Wilks' $F_{14,40} = 11.1$, $P < 0.0001$). In many cases, tadpoles in the lab venue responded more weakly or in the opposite direction to predators than did those outdoors. This was true for lateral RW1, RW3, and RW4: the increasing arch and depth of the tail induced by dragonflies was absent in laboratory tadpoles (Fig. 3). But in other cases, it was the animals in mesocosms that showed a different, and usually greater, response to predators than those in the lab or field. Examples include lateral RW4 and ventral RW2: when exposed to caged predators, mesocosm tadpoles developed a deeper tail, shorter head/body, and narrower gut than did other tadpoles (Fig. 3, Supporting Information C).

Analyses of conventional morphometric lengths generally confirm the results shown here (Supporting Information D).

Precision. – Variation among replicates was not lower in the more controlled venues (Fig. 4). The 1 L laboratory setting had high variance for all types of traits because replicate observations were individuals rather than averages of multiple tadpoles. Other venues and settings had roughly equivalent precision. CV was higher for behavior than for other traits, and did not differ consistently among treatments. Samples from the wild showed relatively low precision.

Discussion

We compared three commonly used experimental venues to evaluate how outcomes depend on venue, and to shed light on constraints and trade-offs associated with choosing a venue. In agreement with previous work, our data show that venue can strongly impact results (Skelly & Kiesecker 2001; Skelly 2002; Bell *et al.* 2003; Brown *et al.* 2006; Romanuk, Vogt & Kolasa 2009; but see Weigensberg & Roff 1996; Blaustein *et al.* 2004; Bancroft *et al.* 2007). We presented two kinds of findings in this study. First, there were strong effects of venue for nearly every trait we measured in developing amphibian larvae, including those related to individual fitness (stage and mass of tadpoles and metamorphs, and survival) and those with a weak or context-dependent connection to fitness (behavior and morphological shape). The venue effect was more pronounced in some traits, such as lateral RW1 and ventral RW1, than in others. The second kind of result was that phenotypic plasticity induced by predation risk depended on venue. Tadpoles in mesocosms exhibited greater behavioral and morphological plasticity, along with larger reductions in performance due to dragonflies, than those in the lab.

It is important to identify the differences among venues that contribute to variation in phenotype and performance, because these might be controlled or incorporated into future experimental designs. For example, variation among venues in development rate was probably related to temperature; tadpoles raised in the laboratory experienced relatively stable and warm temperatures, and therefore developed rapidly. Large body size at emergence in the laboratory, and to a lesser degree in mesocosms, may have been caused by more abundant food of higher quality than was available in field enclosures. Introducing lower-quality food and a diurnally fluctuating temperature regime might create more realistic performance in the lab, although these conditions may conflict with other experimental objectives.

Mesocosms are not natural (Jaeger & Walls 1989; Boone & James 2005), and it has been argued that their unnatural features generate unrealistic experimental outcomes (Carpenter 1996; Skelly 2002). Skelly and Kiesecker (2001; Skelly 2002) have used meta-analyses and experiments to suggest

that amphibian growth and development rates in mesocosms are exceptionally high. But data on performance are problematic for appraising realism of experimental venues, because natural ponds are themselves quite variable. Even our limited sample of three natural ponds encompassed nearly the entire range of variation in growth and development observed across the three venues. Depending on which of the ponds is taken as a reference, we could use Fig. 1 to argue that either field enclosures, mesocosms, or even the laboratory is most realistic. But these differences in performance traits among venues may reflect not realism, but rather variation in temperature or resources that could just as well occur in nature. This argument applies less strongly to morphological shape, which is probably less sensitive to temperature and resources. In this study, tadpoles in the mesocosm settings were morphologically similar to those in field enclosures and natural ponds. This suggests that mesocosms are acceptably realistic for at least morphological traits. Lab-reared tadpoles, on the other hand, were highly divergent in morphology from field and mesocosm specimens.

Our second main finding was that venues differed in the magnitude, and sometimes even the direction, of predator-induced plasticity. In this case, our data do not indicate which venue most closely mirrors nature. However, other studies comparing mesocosm estimates of plasticity with tadpole morphology in natural wetlands having both temporal and spatial variation in predator density indicate that the phenotypic reaction in mesocosms parallels that observed in nature (Van Buskirk & McCollum 1999; Van Buskirk & Schmidt 2000; Van Buskirk 2009). This suggests that, although mesocosms produced relatively high estimates of plasticity in our study, they at least accurately reflect the direction of response seen in nature.

Of the many factors that could cause differences in plasticity among venues and settings, two that seem especially likely are kairomone concentrations and the size of the experimental populations. Within the mesocosm venue, several traits responded more strongly to predators in the 80 L setting, which had higher kairomone concentrations and smaller numbers of tadpoles. Anti-predator reactions are known to scale with kairomone level (Van Buskirk & Arioli 2002; Schoeppner & Relyea 2008), and group size independent of density can modify behavior by affecting individual risk and the perception of risk (Elgar 1989; Van Buskirk *et al.* 2011). In field enclosures, the open mesh walls may have prevented us from successfully manipulating kairomone concentration and apparent predation risk. On the one hand, dilution of kairomones by water flow through the walls could reduce the difference between treatments. Alternatively, all the enclosure animals, including those in the predator-free treatment, may have been exposed to kairomones washed in from nearby wild predators (Chalcraft *et al.* 2005). Indeed, some life history and morphological traits measured in enclosures were most similar to those observed in mesocosms with caged dragonflies (e.g., mass and stage at 24 days, lateral RW1, and ventral RW2), suggesting that kairomone levels may have been high even in the predator-free treatment.

The conclusion that plasticity depends on kairomone concentration was not consistent with results from the laboratory venue. Kairomone concentrations in the lab were identical to those in 80 L mesocosms, yet laboratory animals showed limited plasticity. It is unlikely that additional visual or tactile cues, not present in the lab, amplified the response of animals in mesocosms. Water-borne chemicals induce strong phenotypic plasticity in amphibian larvae (LaFiandra & Babbitt 2004; Kraft *et al.* 2006; Hettyey *et al.* 2010), and adding tactile or visual cues causes no additional reaction, at least for behavior (Stauffer & Semlitsch 1993; Kiesecker, Chivers & Blaustein 1996; Hickman, Stone & Mathis 2004; Jowers *et al.* 2006; Saidapur *et al.* 2009). We suspect instead that unknown features of the laboratory environment bias the expression of behavior and morphology. Identifying precisely which features are important would require further experiments.

This study confirms Skelly and Kiesecker's (2001) conclusion that precision is not greatly enhanced in the lab, contrary to conventional thinking (e.g., Lawton 1995). The 5 L laboratory bins containing groups of five tadpoles showed precision better than that in field enclosures for only one of the two ponds (Fig. 4A), and comparable to that in mesocosms. This result is particularly relevant for the choice of venue because precision is held among the key benefits favoring laboratory work (Lawton 1995; Morin 1998). Our results highlight instead a contrast between experimental setups where units contain small numbers of individuals, leading to relatively high variance among replicates, or large numbers of individuals. More replication is needed when few organisms are present within each replicate.

Our results could be used to defend any of the three venues. For example, the relatively realistic phenotypes exhibited in mesocosms, in combination with the low precision found in small laboratory

bins, argues in favor of more natural experimental settings containing groups of individuals. For most traits, mesocosm experiments apparently yield high realism without sacrificing precision. This conclusion is not affected by the relatively distinct settings occurring in the field venue (different ponds) compared to the mesocosm venue (different sized containers). Precision was calculated as the coefficient of variation among replicates within settings, and realism was judged by comparing experimental with wild tadpoles. Both comparisons are statistically and conceptually identical for all venues and settings, even as the precise meaning of setting changes across venues. Some might conclude that higher phenotypic plasticity in mesocosms indicates that animals overestimate differences in predation risk in that venue. For others, the high variability among enclosures observed within one of the two ponds might argue for using lab or mesocosm venues, especially given other issues with field experiments such as limits on replication and difficulty controlling certain conditions. Overall, the results confirm an age-old recommendation in ecology that the venue must be matched, one study at a time, to the prevailing constraints and the question at hand (Diamond 1986; Wilbur 1989; Morin 1998). Our data will help resolve these trade-offs in specific cases by clarifying the consequences for individual phenotypes.

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References

Bancroft, B.A., Baker, N.J. & Blaustein, A.R. (2007) Effects of UVB radiation on marine and freshwater organisms: a synthesis through meta-analysis. *Ecology Letters*, **10**, 332–345.

Bell, T., Neill, W.E. & Schluter, D. (2003) The effect of temporal scale on the outcome of trophic cascade experiments. *Oecologia*, **134**, 578–586.

Blaustein, L., Kiflawi, M., Eitam, A., Mangel, M. & Cohen, J.E. (2004) Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. *Oecologia*, **138**, 300–305.

Boone, M.D. & James, S.M. (2005) Aquatic and terrestrial mesocosms in amphibian ecotoxicology. *Applied Herpetology*, **2**, 231–257.

Brown, C.J., Blossey, B., Maerz, J.C. & Joule, S.J. (2006) Invasive plant and experimental venue affect tadpole performance. *Biological Invasions*, **8**, 327–338.

Carpenter, S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology*, **77**, 677–680.

Chalcraft, D.R., Binckley, C.A. & Resetarits, W.J. (2005) Experimental venue and estimation of interaction strength: comment. *Ecology*, **86**(4), 1061–1067.

Diamond, J. (1986) Overview: laboratory experiments, field experiments, and natural experiments. *Community ecology* (eds J. Diamond & T.J. Case), pp. 3-22. Harper and Rowe, New York.

Elgar, M.A. (1989) Predator vigilance and group-size in mammals and birds - a critical review of the empirical evidence. *Biological Reviews of the Cambridge Philosophical Society*, **64,** 13–33.

Fraker, M.E., Hu, F., Cuddapah, V., McCollum, S.A., Relyea, R.A., Hempel, J. & Denver, R.J. (2009) Characterization of an alarm pheromone secreted by amphibian tadpoles that induces behavioral inhibition and suppression of the neuroendocrine stress axis. *Hormones and Behavior*, **55**, 520–529.

Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183–190.

Hairston, N.G. (1989) *Ecological experiments: purpose, design, and execution*. Cambridge.

Hettyey, A., Zsarnoczai, S., Vincze, K., Hoi, H. & Laurila, A. (2010) Interactions between the information content of different chemical cues affect induced defences in tadpoles. *Oikos*, **119**, 1814–1822.

Hickman, C.R., Stone, M.D. & Mathis, A. (2004) Priority use of chemical over visual cues for detection of predators by graybelly salamanders, *Eurycea multiplicata griseogaster*. *Herpetologica*, **60**, 203–210.

Jaeger, R.G. & Walls, S.C. (1989) On Salamander Guilds and Ecological Methodology. *Herpetologica*, **45**, 111–119.

Jowers, M.J., Campell-Palmer, R., Walsh, P.T. & Downie, J.R. (2006) Intraspecific variation in the avoidance response of stream frog (*Mannophryne trinitatis*) tadpoles to fish and prawn predators. *Herpetological Journal*, **16**, 337–346.

Kiesecker, J.M., Chivers, D.P. & Blaustein, A.R. (1996) The use of chemical cues in predator recognition by western toad tadpoles. *Animal Behaviour*, **52**, 1237–1245.

Kraft, P.G., Franklin, C.E. & Blows, M.W. (2006) Predator-induced phenotypic plasticity in tadpoles: extension or innovation? *Journal of Evolutionary Biology*, **19**, 450–458.

LaFiandra, E.M. & Babbitt, K.J. (2004) Predator induced phenotypic plasticity in the pinewoods tree frog, *Hyla femoralis*: necessary cues and the cost of development. *Oecologia*, **138**, 350–359.

Lawton, J.H. (1995) Ecological Experiments with Model Systems. *Science*, **269**, 328–331.

Morin, P.J. (1998) Realism, precision, and generality in experimental tests of ecological theory. *Issues and perspectives in experimental ecology* (eds W.J. Resetarits & J. Bernardo), pp. 50-70. Oxford University Press.

Pearman, P.B. (1993) Effects of habitat size on tadpole populations. *Ecology*, **74**, 1982-1991.

Petersen, J.E., Cornwell, J.C. & Kemp, W.M. (1999) Implicit scaling in the design of experimental aquatic ecosystems. *Oikos*, **85**, 3–18.

Romanuk, T.N., Vogt, R.J. & Kolasa, J. (2009) Ecological realism and mechanisms by which diversity begets stability. *Oikos*, **118**, 819–828.

Saidapur, S.K., Veeranagoudar, D.K., Hiragond, N.C. & Shanbhag, B.A. (2009) Mechanism of predator-prey detection and behavioral responses in some anuran tadpoles. *Chemoecology*, **19**, 21–

28.
Schiesari, L. (2006) Pond canopy cover: a resource gradient for anuran larvae. *Freshwater Biology*, **51**, 412–423.
Schoeppner, N.M. & Relyea, R.A. (2008) Detecting small environmental differences: risk-response curves for predator-induced behavior and morphology. *Oecologia*, **154**, 743–754.
Skelly, D.K. (2002) Experimental venue and estimation of interaction strength. *Ecology*, **83**, 2097–2101.
Skelly, D.K. & Kiesecker, J.M. (2001) Venue and outcome in ecological experiments: manipulations of larval anurans. *Oikos*, **94**, 198–208.
Skelly, D.K., Freidenburg, L.K. & Kiesecker, J.M. (2002) Forest canopy and the performance of larval amphibians. *Ecology*, **83**, 983–992.
Spivak, A.C., Vanni, M.J. & Mette E.M. (2011) Moving on up: can results from simple aquatic mesocosm experiments be applied across broad spatial scales? *Freshwater Biology*, **56**, 279–291.
Stauffer, H.P. & Semlitsch, R.D. (1993) Effects of visual, chemical and tactile cues of fish on the behavioral responses of tadpoles. *Animal Behaviour*, **46**, 355–364.
Urban, M.C. (2008) The evolution of prey body size reaction norms in diverse communities. *Journal of Animal Ecology*, **77**, 346–355.
Van Buskirk, J. (2009) Natural variation in morphology of larval amphibians: phenotypic plasticity in nature? *Ecological Monographs*, **79**, 681–705.
Van Buskirk, J. (2011) Amphibian phenotypic variation along a gradient in canopy cover: species differences and plasticity. *Oikos*, **120**, 906–914.
Van Buskirk, J. & Arioli, M. (2002) Dosage response of an induced defense: how sensitive are tadpoles to predation risk? *Ecology*, **83**, 1580–1585.
Van Buskirk, J. & McCollum, S.A. (1999) Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. *Oikos*, **85**, 31–39.
Van Buskirk, J. & Schmidt, B.R. (2000) Predator-induced plasticity in larval newts: trade-offs, selection, and variation in nature. *Ecology*, **81**, 3009–3028.
Van Buskirk, J., Ferrari, M., Küng, D., Naepflin, K. & Ritter, N. (2011) Prey risk assessment depends on conspecific density. *Oikos*, **120**, 1235-1239.
Weigensberg, I. & Roff, D.A. (1996) Natural heritabilities: Can they be reliably estimated in the laboratory? *Evolution*, **50**, 2149–2157.
Wilbur, H.M. (1989) In defense of tanks. *Herpetologica*, **45**, 122–123.
Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. (2004) *Geometric morphometrics for biologists*. Elsevier, New York.

Table 1. Summary of the experimental venues and settings. The concentration of kairomones in the caged-predator treatment is reported in two ways: the mass of live tadpoles consumed per volume and per week, and the density of dragonflies per volume. For wild tadpoles, the table lists the area and volume of the three ponds at maximum depth, and the number of tadpoles sampled from each pond.

Venue	Setting	No. of replicates	Surface area (m^2)	Volume (L)	No. of tadpoles	No. of caged *Anax*	Kairomone concentration	
							mg tad/L/week	*Anax*/L
Laboratory	Small bin	10	0.0228	1.1	1	--	11.25	0.0125
Laboratory	Large bin	6	0.06	5.2	5	--	11.25	0.0125
Mesocosm	Small tub	5	0.28	80	12	1	11.25	0.0125
Mesocosm	Large tank	4	1.35	675	60	2	2.67	0.0030
Field enclosure	Sunny pond	3	1.0	200	35	1	4.50	0.0050
Field enclosure	Shady pond	3	1.0	200	35	1	4.50	0.0050
Wild tadpoles	Source pond	1	2500	6.2×10^5	9	--	--	--
Wild tadpoles	Sunny pond	1	650	2.0×10^5	10	--	--	--
Wild tadpoles	Shady pond	1	300	1.0×10^5	10	--	--	--

Table 2. Results of univariate analyses of variance on life history, behavior, and morphological shape in lateral and ventral view. Entries are the *F*-value (above) and *P*-value (below). Numerator df are listed at the top, and the denominator for all effects was the interaction between treatment and block nested within setting and venue (df = 26). For activity, df were reduced because there were only two venues (lab and mesocosm). Bold text highlights tests significant at $\alpha = 0.05$. Masses are log-transformed, survival and behavioral traits are arcsin-sqrt transformed, and shape components are relative warps derived from geometric morphometric analyses.

	Source of variation				
Response variable	Treatment (df=1)	Venue (df=2)	Setting(Venue) (df=3)	Treatment × Venue (df=2)	Treatment × Setting(Venue) (df=3)
Life history traits					
Tadpole mass	**5.55**	**188.01**	1.30	**16.53**	**4.62**
at age 24 days	**0.0263**	**<0.0001**	0.2961	**<0.0001**	**0.0101**
Developmental stage	**5.10**	**113.49**	1.90	**9.28**	2.61
at age 24 days	**0.0326**	**<0.0001**	0.1542	**0.0009**	0.0731
Survival to	0.00	**47.89**	2.69	0.06	0.47
metamorphosis	0.9772	**<0.0001**	0.0672	0.9393	0.7041
Mass at	2.56	**125.28**	**3.39**	**8.63**	**4.71**
metamorphosis	0.1214	**<0.0001**	**0.0330**	**0.0013**	**0.0094**
Age at	**8.95**	**264.92**	**3.78**	**9.58**	0.45
metamorphosis	**0.0060**	**<0.0001**	**0.0225**	**0.0008**	0.7192
Behavior at age 18 days					
Activity	**84.13**	**6.14**	**4.99**	**56.73**	**10.04**
(hiding not counted)	**<0.0001**	**0.0234**	**0.0189**	**<0.0001**	**0.0012**
Activity	**40.15**	**23.49**	**3.82**	**21.04**	2.50
(hiding are inactive)	**<0.0001**	**0.0001**	**0.0415**	**0.0002**	0.1105
Morphological shape components at age 24 days					
lateral RW1	1.01	**263.13**	**6.32**	**8.73**	0.60
	0.3237	**<0.0001**	**0.0023**	**0.0013**	0.6194
lateral RW2	2.53	**8.14**	1.50	1.35	0.29
	0.1235	**0.0018**	0.2387	0.2758	0.8339
lateral RW3	**12.76**	2.00	2.97	**9.45**	0.50
	0.0014	0.1551	0.0502	**0.0008**	0.6867
lateral RW4	**39.64**	**4.13**	**7.31**	**14.68**	1.35
	<0.0001	**0.0276**	**0.0010**	**<0.0001**	0.2793
ventral RW1	0.04	**216.20**	0.22	3.26	0.69
	0.8342	**<0.0001**	0.8830	0.0545	0.5684
ventral RW2	**39.52**	**18.76**	**5.58**	**51.74**	**8.22**
	<0.0001	**<0.0001**	**0.0043**	**<0.0001**	**0.0005**
ventral RW3	**7.87**	**27.00**	2.80	**12.80**	0.10
	0.0094	**<0.0001**	0.0601	**0.0001**	0.9588

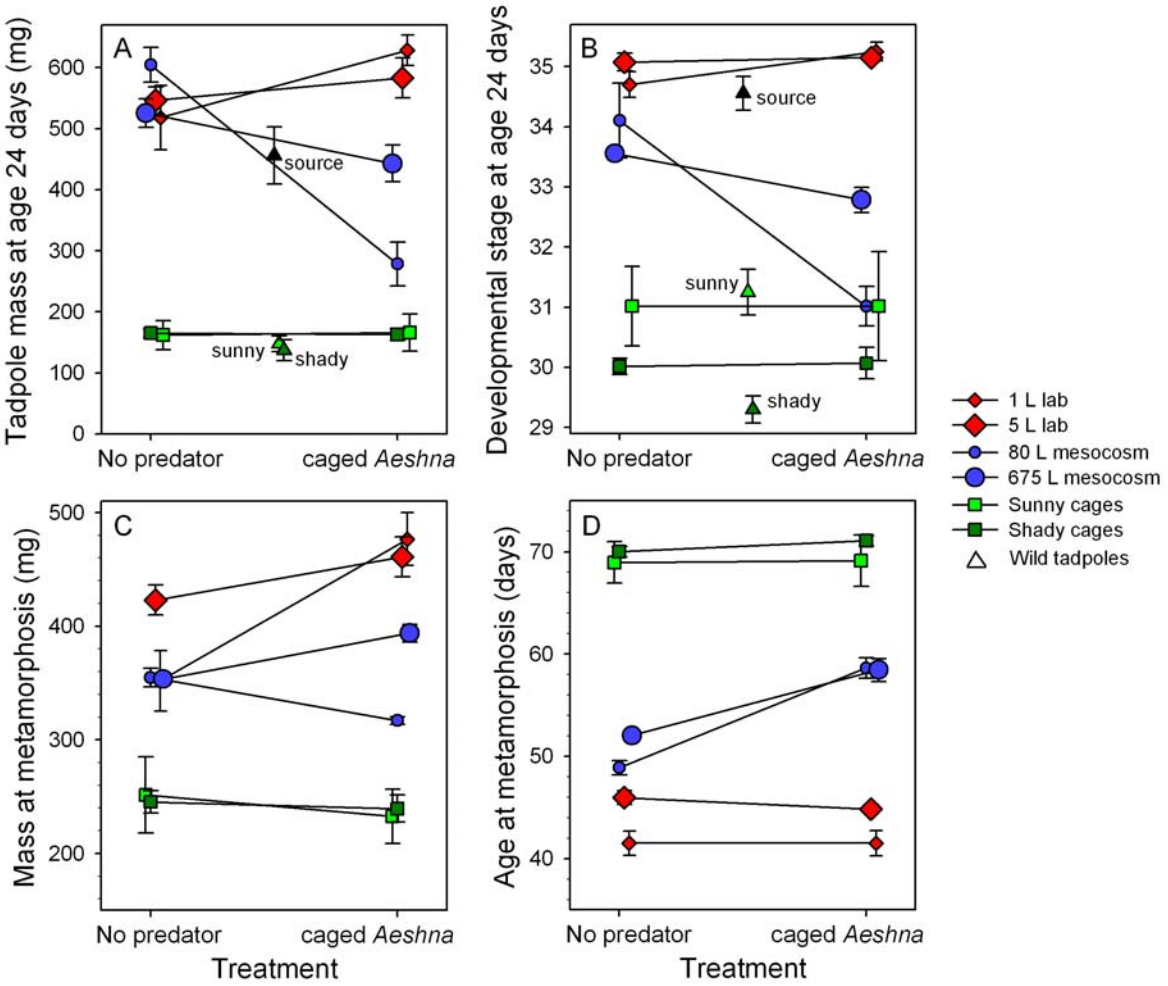

Figure 1. Life history responses of *Rana temporaria* tadpoles to manipulation of venue and non-lethal predation risk (treatment). Developmental stage is defined in Gosner (1960). Metamorphic mass and age were measured at stage 45, after the tail was fully resorbed. Symbols represent means ± 1 SE.

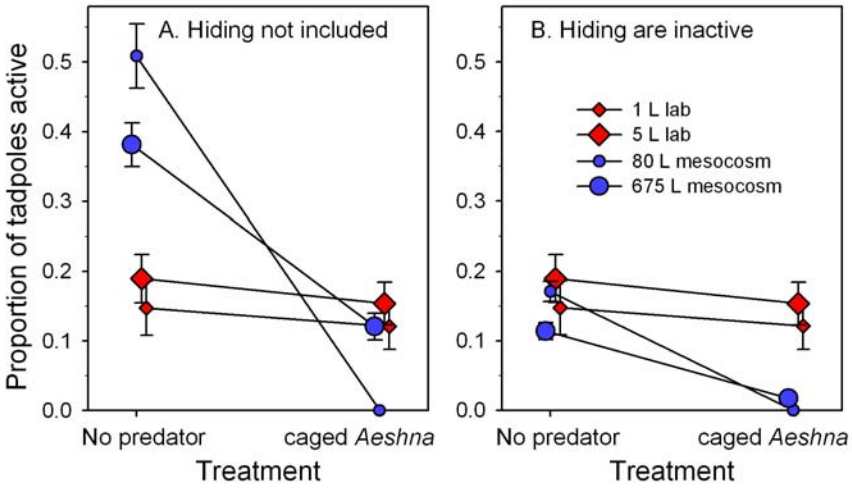

Figure 2. *Rana temporaria* tadpole activity measured at age 18 days. Symbols represent means ± 1 SE of the proportion of visible tadpoles that were active (A) or the proportion of tadpoles active under the assumption that hiding individuals were inactive (B).

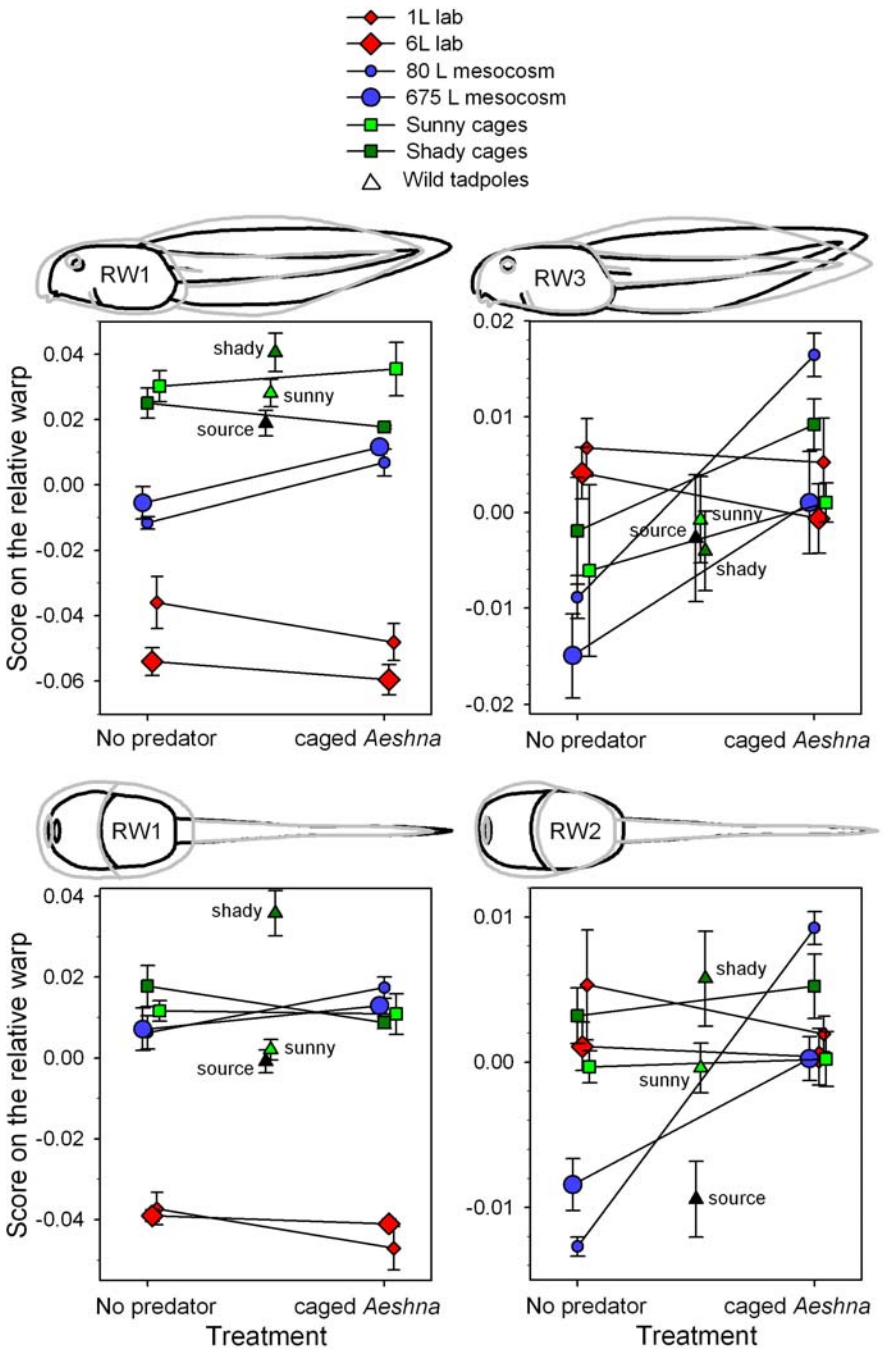

Figure 3. Morphological shape components measured at 24 days of age. Tadpole drawings illustrate shape changes represented by relative warps: the gray/black outlines show tadpoles with scores 2 SD above/below the mean form (lateral RW1 and ventral RW1), 3 SD above/below the mean (lateral RW3), or 4 SD above/below the mean (ventral RW2). Error bars indicate ± 1 SE based on replicate tubs (experiment) or individual tadpoles (wild samples).

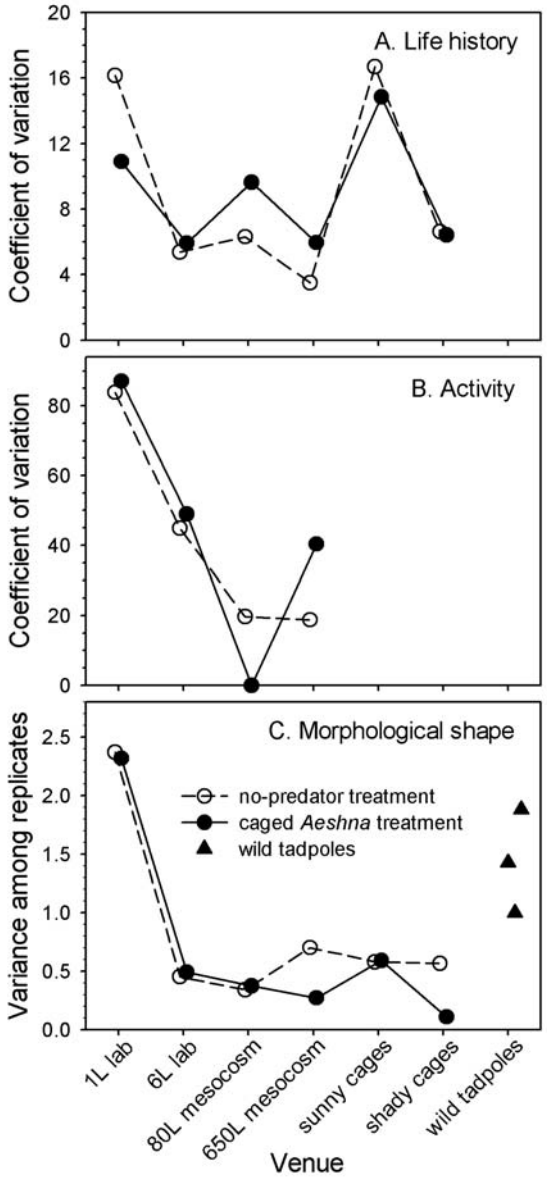

Figure 4. Precision of the estimate of tadpole life history, behavior, and morphology in each venue. Points show the average coefficient of variation (A, B) or variance (C) among replicates. The numbers of traits included are 5 in A (Fig. 1 and survival), 2 in B (Fig. 2), and 7 in C (lateral RW1-4 and ventral RW1-3). Variance components for morphological traits were multiplied by 104. The sequence of venues along the horizontal axis is approximately in decreasing order of experimental control and increasing order of ecological complexity. Low values of variation among replicates indicate high precision.